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Dual-Modal Tumor Imaging via Long-Circulating Biodegradable Core-Crosslinked Polymeric Micelles

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Abstract

We present a long-circulating biodegradable core-crosslinked polymeric micelle (d-CCPM) for the nuclear/optical imaging of tumors. The d-CCPM was derived from an amphiphilic block-copolymer consisting of a hydrophilic block of brush-like poly(ethylene glycol) and a hydrophobic block containing cleavable pendent triethoxysilane. The resultant imaging tracer had prolonged circulation in the blood (half-life of clearance phase = 36.5 hrs), substantial accumulation in tumor (% injected dose per gram of tissue = $8.5\% \pm 1.0\%$ at 24 hrs post-injection) and minimal uptake in the liver ($5.0\% \pm 0.1\%$) or spleen ($5.1\% \pm 0.3\%$). Both nuclear and near-infrared fluorescence imaging revealed strong signals in tumor regions. At 48 hrs, nuclear imaging exhibited tumor-to-liver and tumor-to-blood ratios of 1.4 and 1.1, respectively. The degradation of d-CCPM was studied *in vitro* at pH 5.0 and 37°C; and confirmed by transmission electron microscopy confirmed. Our study indicates that the d-CCPM system is an effective probe for dual-modal cancer imaging and a potential safe platform nanocarrier for the delivery of anti-cancer drugs and cancer therapy.

Keywords

Polymeric Micelles; Core Crosslinked; Tumor Imaging; Near-infrared Fluorescence; γ -Scintigraphy

Single-modal imaging tracers can hardly meet the complex requirements of modern tumor imaging, such as target specificity, high sensitivity, high spatial resolution, sufficient tissue penetration and 3-dimensional tomography¹. Multiple imaging tracers incorporated into one nanoparticle, on the other hand, are able to integrate the merits of individual components and compensate for their deficiencies. The nanoparticle platform can be further tailored to obtain desirable pharmacokinetics and minimal non-tumor uptake by optimizing the size and surface properties². The passive accumulation of nanoparticles in tumor is achieved via the enhanced permeation and retention (EPR) effect due to the leaky vascular structures and lack of lymphatic drainage in tumor tissue³. The tumor uptake of nanoparticles can be augmented using targeting ligands such as folic acid and some tumor-specific peptides^{4–7}. Nevertheless, a prolonged circulation is still favorable because it increases the residential

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ASSOCIATED CONTENT

Supporting information is available free of charge via the Internet at <http://pubs.acs.org/journal/amlccd>

time of targeting ligands that pass through the tumor and become recognized by the targets⁸. However, many imaging tracers currently under investigation still exhibited a short circulation half-life; their entrapment in the liver and spleen consumed a significant part of injected dose^{9–13}.

Recently we reported a core-crosslinked polymeric micelle (CCPM) system with a prolonged blood circulation and low uptake in the liver or spleen¹⁴. These qualities were probably due to the crosslinked core that prevented the premature micelle disintegration *in vivo*. Also, the brush-like PEG formed a dense protective layer on the micelle surface and minimized the micelle uptake in the reticuloendothelial system (RES). Lastly, the micelle size (24 ± 8.9 nm) was above the threshold of renal clearance yet not so large as to be captured by RES¹⁵. However, this CCPM lacked biodegradability and might pose health hazard for the long-term applications. Degradable polymeric micelles have been developed by incorporating cleavable linkages into the polymer backbone or pendent groups^{16, 17}.

In this study we successfully introduced biodegradability into the crosslinker of CCPM system without compromising its merits in biodistribution, pharmacokinetics and imaging quality. The preparation of this degradable CCPM (d-CCPM) is illustrated in (Scheme 1). The polymer precursor consisted of a hydrophilic block of brush-like PEG, as well as a hydrophobic block containing pendent triethoxysilane through a degradable succinic ester bond. Micelles were formed spontaneously upon the slow addition of water into the methanol solution of the copolymer and a NIRF fluorophore, 3-triethoxysilyl-propyl IR783 (NIRFSi). The crosslinking followed the hydrolysis and condensation of triethoxysilane, while NIRFSi was simultaneously loaded into d-CCPM at 0.2 % wt. The d-CCPM was then purification via dialysis and filtration. The fluorescence emission spectrum of d-CCPM in PBS (pH 7.4) exhibited a maxima at 831 nm ($\lambda_{ex} = 765$ nm, see Figure S3 in supporting information). The surface amine of d-CCPM was conjugated to a metal chelator, diethylenetriamine pentaacetic acid (DTPA), to enable the radiolabeling with ¹¹¹In at 195 μ Ci per mg of micelles. The radiolabeling efficiency was 94% (Figure S5). The radiolabeling was quite stable. When ¹¹¹In-labeled d-CCPM was incubated in mouse whole blood at 37 °C, no significant dissociation of ¹¹¹In from d-CCPM was observed after 7 days of incubation (Figure S6).

The average hydrodynamic diameter of d-CCPM was 25.2 nm with a narrow size distribution (PDI = 0.037, Figure 1A). Similar results were observed in the transmission electron microscopy (TEM) image (Figure 1B). The micelles were hydrolyzed by the scission of succinic ester bonds between the silica clusters and the surrounding polymer backbones (see the inset of Scheme 1). Succinic ester bond is known to be susceptible to hydrolysis in acidic environments, e.g. lysosomes^{18–20}. Disintegrated d-CCPM was observed after 1 week of incubation in a pH 5.0 buffer at 37 °C (Figure 1C). In contrast, the non-degradable CCPM, in which the pendent triethoxysilane was linked through a non-degradable bond to the backbone of the hydrophobic block, remained intact under a harsher condition (0.1 M HCl, Figure 1D).

The d-CCPM had low cytotoxicity to human liver carcinoma (Hep-G2) and human embryonic kidney 293 (HEK-293) cell lines. No significant toxicity was found in either cell line as the micelle concentration increased from 10 to 1000 μ g/ml (Figure 2).

All animal studies were carried out in accordance to institutional animal care and use guidelines. *In vivo* pharmacokinetics was evaluated in female BALB/c mice using ¹¹¹In-labeled d-CCPM. The blood activity-time profile (Figure 3A) fits well into a two-compartment model described by the equation²¹:

$$Ct (\%ID/g) = Ae^{-\alpha t} + Be^{-\beta t}$$

Based on this equation, the half-life of ^{111}In -labeled d-CCPM was 0.5 hr in the distribution phase and 36.5 hrs in the clearance phase.

Biodistribution data were obtained from nude mice bearing subcutaneous CT-26 tumors at 24 and 48 hrs post-injection (Figure 3B). The d-CCPM level (%ID/g) in the blood was 15.4 ± 1.5 at 24 hrs and decreased to 7.5 ± 0.8 at 48 hrs. RES organs had low uptake at both time points. The liver uptake was 5.0 ± 0.1 and 6.1 ± 0.4 , while the spleen uptake was 5.1 ± 0.3 and 6.8 ± 0.3 , respectively. CCPM accumulation in tumor at 24 hrs was 8.5 ± 1.0 and did not change at 48 hrs (8.5 ± 1.7). Most notably, at 48 hrs, more d-CCPM resided in tumor than blood, liver or spleen, underscoring substantial reduction in RES uptake of d-CCPM. The autoradiograph and fluorescence scanning of tumor slices demonstrated the colocalization of both imaging tracers, suggesting that over the period of the 48-hr study both the ^{111}In -labeled backbone and NIRFSi-labeled crosslinking core remained associated with residual d-CCPM (Figure S7). Biodistribution data at 72 and 96 hrs were also recorded (Figure S8). Compared to the results at shorter time, there was significant increase in liver uptake (%ID/g): 16.7 ± 0.4 at 72 hrs and 15.6 ± 1.5 at 96 hrs. Such increase indicated that degraded d-CCPM was captured by liver. Uptakes in the spleen, 4.2 ± 0.2 at 72 hrs and 4.3 ± 0.3 at 96 hrs, were lower than at earlier time points. Notably, most d-CCPM was cleared from the lung by 72 hrs. The lung uptake was 0.6 ± 0.1 at both 72 and 96 hrs, while lung uptakes were 7.1 ± 0.5 and 5.6 ± 0.2 at 24 and 48 hrs, respectively.

Radioactivity was detected in both urine and feces post injection. Considering the excellent stability of radiolabeling, such radioactivity in urine and feces indicated the clearance of d-CCPM (Figure 3C). The significant urine clearance during the first 6 hrs (11.3% of injected dose excreted). Similar finding was recorded with non-degradable CCPM¹⁴. The renal clearance threshold for soft d-CCPM may be higher than hard quantum dots, which was previously determined to be around 5 nm in diameter¹⁵. The clearance via feces suggested that d-CCPM was degraded in liver and excreted through bile. The clearance study was stopped at 96 hrs due to the short half-life of ^{111}In . Long-term clearance would require isotopes with longer half-lives.

Figure 4 shows representative images from γ -scintigraphy, single-photon-emission computed tomography (SPECT) and NIRF at different time intervals after *i.v.* injection of ^{111}In -labeled d-CCPM. Micelles were administrated at a dose of 1.0 mg/mouse, corresponding to 195 μCi ^{111}In and 2.1 nmol fluorophore per mouse. The γ -imaging (Figure 4A) results were concordant with the biodistribution data. At 2 hrs, the blood pool emitted strong signals. The high-uptake areas were the lung, liver and spleen. The activity from blood pool and background decreased over time while d-CCPM accumulated in tumor. The tumor was clearly visualized by 24 hrs post-injection. By 48 hrs post-injection, there was higher uptake in the tumors than in the liver and the spleen. SPECT mapped the 3-dimensional distribution of d-CCPM, which was consistent with the γ -imaging results. NIRF also showed that the tumor uptake increased from 2 hrs to 24 hrs and remained the same at 48 hrs (Figure 4C).

In conclusion, we successfully developed a dual-modal imaging tracer using d-CCPM that exhibited degradability, minimal cytotoxicity, prolonged blood circulation, reduced RES uptake and significant accumulation in tumor. Our study proves that d-CCPM system is an effective probe for tumor imaging and a potentially safe platform nanocarrier for drug delivery and cancer therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (A) Dynamic light scattering (DLS) histogram and (B) TEM image of d-CCPM prior to degradation; (C) TEM images of disintegrated d-CCPM in pH 5.0 buffer and (D) non-degradable CCPM after 1 week of incubation at 37 °C in 0.1 M HCl.

Figure 2.

In vitro cell toxicity study of CCPM-NIRFSi using human embryonic kidney (HEK-293) and human liver carcinoma Hep-G2 cell lines. Untreated cells were used as control. Viability data were normalized against the control groups. All data were presented as mean $\pm$ standard deviation ($n = 6$). The standard deviation values were too small to be visible in the figure.

Figure 3.

Pharmacokinetics, biodistribution and whole body clearance studies. (A): blood activity-time profile. Filled circles represent the mean radioactivity expressed as % injected dose per gram of blood (%ID/g) from 10 mice. The solid line is a curve fitted to a two-compartment model; (B): biodistribution results obtained from radioactivity count (4 mice each time point), plotted as %ID per gram of tissue. (C): whole body clearance via urine (open diamond) and feces (filled circle) at each time point were collected from 6 mice, plotted as % injected dose. All data are expressed as mean $\pm$ standard error.

Figure 4. γ -scintigraphy (A), SPECT (B) and NIRF (C) images post injection of ^{111}In -d-CCPM. Mice were at dorsal positions in (A) and ventral positions in (C).

Scheme 1.

Synthesis and degradation of CCPM loaded with radioisotope ^{111}In and NIRF dye. Detailed structure of the crosslinking silica cluster is illustrated in the inset.